REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Claims 1-16 and 19-35 are in this case.

Claims 5 and 23-35 were withdrawn by the Examiner from consideration as drawn to a non-elected invention.

Claims 1,3 and 7-11 have been rejected under 35 U.S.C. §112, second paragraph.

Claims 1,3,6-13, 15 and 22 have been rejected under 35 U.S.C. §102(b).

Claims 1,3,6-13, 15 and 22 have been rejected under 35 U.S.C. §102 (a).

Claims 1,3,4,6-15 and 19-22 have been rejected under 35 U.S.C. §103 (a).

Dependent claims 3, 9, 10 and 11 have been amended. Amendments are purely linguistic and do not introduce new matter.

The claims before the Examiner are directed toward vectors for expressing heterologous peptides at the amino-terminus of Potyvirus Coat Protein, methods for use thereof, plants infected with same and methods of vaccination using same.

§ 112, Second Paragraph Rejections

The Examiner has rejected claims 1,3,7-11 and under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

Specifically, the Examiner has asserted that claims 1,3,10 and 11 are indefinite because of disagreement between "a heterologous nucleic acid" and "at least a portion of the heterologous peptide". Claims 3, 10 and 11 have been amended so that the antecedent basis

of heterologous peptide is absolutely clear. These amendments are purely linguistic and no introduction of new matter has occurred.

The Examiner's rejection of claims 1,3,10 and 11 under 35 U.S.C. §112, second paragraph is traversed.

The Examiner has further asserted that claims 1,7 and 8 are indefinite because of the phrase "at least one amino acid residue". The Applicant respectfully invites the Examiner to read the three claims in question more closely. Claim 1, as the independent claim, is necessarily the broadest in scope and does not require the presence of an amino terminal domain of the coat protein. Applicant stresses that "amino-terminus" is <u>not</u> synonymous with "amino-terminal domain".

The term "terminus" was specifically employed to designate the extreme end of the peptide. Such usage is consistent with the dictionary definition of "terminus:" which is "an end; final point; extremity or goal" [Webster's New World Dictionary; Second College Edition (1976) William Collins & World Publishing Inc; D.B. Guralnik (editor)].

Further, the term "terminus", whether amino- or carboxy-, is commonly employed by those of ordinary skill in biochemistry to denote an end of a peptide or protein.

Thus, the choice of the term "amino-terminus" was made to "to particularly point out and distinctly claim the subject matter which Applicant regards as the invention" as required by 35 U.S.C. §112, second paragraph.

Claim 7 depends from claim 1 and limits the scope thereof so that the vector is defined, for the first time, as including an "amino-terminal domain". Again, "amino-terminal domain", as opposed to "amino-terminus", is readily understood by those of ordinary skill in the art of virology of potyviridae. Applicant has provided, solely in order to expedite prosecution, pages 121-127 of the standard reference text "Shukla, D.D., Ward, C.W. & Brunt, A.A.; <u>The Potyviridae</u> (1994) Wallingford,UK, CAB International.516 p." [see Appendix A]. The

Examiner is specifically referred to page 121 and to Table 5.1 in which AA sequences of the amino-terminal domain, including the amino-terminus of several potyviruses including ZYMV are set forth. Applicant respectfully points out that since this standard text was widely available more than seven years prior to the filing date of the instant application, it is reasonable to assume that one of ordinary skill in the art would understand the terminology as used therein.

Applicant notes that, owing to an earlier restriction requirement, the potyvirus of the vector is ZYMV. The portion of ZYMV which comprises the amino-terminal domain is well known to those of ordinary skill in the art for many years as set forth hereinabove.

Thus, claim 8, which further limits claim 7 by stating "...wherein said aminoterminal domain is modified by deletion of at least 1 amino acid residue." includes a vector in which any number of residues are deleted so long as at least one residue of the recognized amino terminal domain remains. Because the definition of aminoterminal domain is concrete in the mind of those ordinarily skilled in the art, the language of claim 8 is not indefinite.

In summary, claim 1 includes both vectors with an amino-terminal domain and also those that lack such a domain. Claim 7 includes only those vectors that include an amino-terminal domain. Claim 8 makes it clear that deletions from the amino-terminal domain do not remove the vector from the scope of the claims. For the record, Applicant states that as long as one amino acid residue of the amino-terminal domain remains, the vector is claimed under claims 1, 7 and 8. If no amino acid residue of the amino-terminal domain remains, the vector is claimed under claim 1. If the entire amino-terminal domain is present, the vector is claimed under claims 1 and 7.

The Examiner's rejection of claims 1, 7 and 8 under 35 U.S.C. §112, second paragraph is traversed.

The Examiner has further asserted that claims 1,3 and 9 are indefinite because of the phrase "influences a biological activity". The Examiner asserts that it is unclear that it is the biological activity of the [at least a portion of the] heterologous peptide that is modified. Such an assertion is untenable in the face of the language of claim 9, which is completely unambiguous in this regard. Claims 1 and 3 do not contain the [allegedly] indefinite phrase.

The Examiner's rejection of claims 1, 3 and 9 under 35 U.S.C. §112, second paragraph is traversed.

All rejections under 35 U.S.C. §112, second paragraph are traversed.

§ 102(b) Rejections - Fernandez-Fernandez

The Examiner has rejected claims 1,3,6-13,15 and 22 under §102(b) as being anticipated by Fernandez-Fernandez et al. (Federation of European Biochemical Societies, 1998; hereinafter Fernandez).

The Applicant stresses that Fernandez teaches insertion of heterologous sequence(s) between coat protein residues Ala₁₂ and Leu ₁₃. Thus Fernandez does not teach, hint or fairly suggest that insertion of a heterologous sequence at the amino-terminus, as instantly claimed, is necessary, advantageous, desirable or even feasible. Arguments concerning the meaning of "amino-terminus" are set forth in detail hereinabove. By definition, any insertion of "heterologous sequence(s) between coat protein residues Ala₁₂ and Leu ₁₃" will not be at the "amino-terminus" as instantly claimed.

The Applicant was aware of Fernandez earlier work which is reviewed on page 5 of the specification as filed. Applicant reiterates that "This [Fernandez's] insertion did not

involve a deletion of any part of the PPV authentic CP-NT nor was the heterologous peptide fused to the extreme N- terminus."

By contrast, the claims before the Examiner are limited to those vectors which include "a heterologous nucleic acid sequence inserted at the amino-terminus of the potyvirus coat protein." [claim 1]. Applicant stresses that amino terminal domain and amino-terminus are <u>not</u> synonymous as described in detail hereinabove. See also claim 2 and page 8; last paragraph:

"According to further features in preferred embodiments of the invention described below, the <u>amino-terminus</u> is selected from the group consisting of: (i) an established <u>amino-terminus</u> of a wild type potyvirus coat protein; and (ii) an alternate <u>amino-terminus</u> of a potyvirus coat protein, the alternate <u>amino-terminus</u> arising from an action selected from the group consisting of an insertion, a replacement and a deletion of at least one amino acid residue from the known <u>amino-terminus</u>."

Further, owing to an earlier restriction requirement in this case, the scope of claims 1-22 has been limited to ZYMV. Fernandez's teachings deal with plum pox virus (PPV). PPV appeared in claim 5, which has been withdrawn. The Examiner has, on the one hand attempted to exclude PPV from what is claimed while, on the other hand, relied upon a PPV research article to formulate an anticipation rejection.

Applicant respectfully asserts that such a practice is not proper.

The Examiner's § 102(b) rejection based upon Fernandez is traversed.

§ 102(a) Rejections - Varrelmann

The Examiner has rejected claims 1,2,3,6-9,15 and 22 under §102(a) as being anticipated by Varrelmann et al. (Journal of Virology, 2000; hereinafter Varrelmann)

The objective of Varrelmann is to demonstrate the feasibility of mutating the <u>core</u>

<u>domain</u> of a coat protein in a potyvirus (see Figure 1 of Varrelmann). Thus, Varrelmann, like

Fernandez, does not teach, hint or fairly suggest that insertion of a heterologous sequence at the amino-terminus, as instantly claimed, is necessary, advantageous, desirable or even feasible.

Again, the claims before the Examiner are limited to those vectors which include "a heterologous nucleic acid sequence inserted at the amino-terminus of the potyvirus coat protein." [claim 1]. Applicant stresses that the claimed amino-terminus does not reside within the "core" of the CP as taught by Varrelmann..

Further, owing to an earlier restriction requirement in this case, the scope of claims 1-22 has been limited to ZYMV. Varrelmann's teachings deal with plum pox virus (PPV). PPV appeared in claim 5, which has been withdrawn. The Examiner has, on the one hand attempted to exclude PPV from what is claimed while, on the other hand, relied upon a PPV research article to formulate an anticipation rejection.

Applicant respectfully asserts that such a practice is not proper.

The Examiner's §102(a) rejection based upon Varrelmann is traversed.

All §102 rejections are traversed.

§ 103(a) Rejections - Fernandez and others

The Examiner has rejected claims 1,3,4,6-15 and 19-22 under §103(a) as being obvious with respect to Fernandez in view of US 5,955,647 (hereinafter Fitchen) and further in view of Atreya et al. (PNAS, 1993; hereinafter Atreya).

The Applicant reiterates, owing to an earlier restriction requirement in this case, the scope of claims 1-22 has been limited to ZYMV. Fernandez's teachings deal with plum pox virus (PPV). PPV appeared in claim 5, which has been withdrawn.

Similarly, Fitchen teaches mutation of TMV. TMV is not a potyvirus. As such, any inference that what is true for TMV will be true for ZYMV is not valid. Further, Fitchen teaches modification of the amino-terminal domain, not the amino-terminus, as set forth hereinabove in relation to Fernandez and to Varrelmann.

Further, Atreya fails to teach "a heterologous nucleic acid sequence inserted at the amino-terminus of the potyvirus coat protein." as instantly claimed.

Further, the Examiner has attempted to limit the claims to ZYMV while relying on non-ZYMV citations to formulate an obviousness rejection. Applicant respectfully asserts that such a practice is not proper, especially as regards the Fitchen reference which deals with a virus outside the potyvirus family.

In summary, none of the references hint or fairly suggest, whether alone or in combination, what is claimed.

The Examiner's rejection under §103(a) is traversed.

All rejections are traversed.

MPEP § 821.0-Right to Rejoinder

Applicant respectfully asserts that independent claims 23, 26 and 28, currently withdrawn, include all of the limits of claim 1. Because claim 1 is in condition for allowance, rejoinder of these withdrawn claims, and all claims which depend therefrom, is respectfully requested.

In view of the above amendments and remarks it is respectfully submitted that

independent claims 1, and hence dependent claims 2-15 and 19-22 are in condition for

allowance. Prompt notice of allowance is respectfully and earnestly solicited. Further,

rejoinder of claims 23-35, and their allowance, is respectfully requested.

Respectfully submitted,

Mark M. Friedman

Attorney for Applicant

Registration No. 33,883

Date: August 16, 2004

14

Dharma D. Shukla

CSIRO

Division of Biomolecular Engineering

343 Royal Parade MELBOURNE

Australia Victoria 3052

Colin W. Ward

CSIHO

Division of Biomolecular Engineering 343 Royal Parade

Victoria 3052 MELBOURNE

Australia

pma

Alan A. Brunt

Worthing Hoad Horticulture Research International

West Sussex BN17 6LP NO.14INVIEW.

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Cout Protein Structure and Variation

sequences are summarized in Table 5.1 along with the corresponding reference citations and information on the coat protein size and the length of the 3' non-coding region. In addition, comparative coat protein sequence analyses of 96 strains of 25 viruses have been made by high performance liquid chromatographic (HPLC) profiling of tryptic peptides combined with amino acid composition and sequence analysis of selected peptides (Table 5.2). This approach enables the sequence identity between groups of coat proteins to be estimated rapidly without resorting to the rigours of full protein sequence determination.

One foature that has frustrated amino acid sequence determinations of potyvirus coat proteins has been the presence of an N-torminal blocking group on some coat proteins but not othors. Blocked N-terminal residues have been found for JGMV (Shukla et al., 1987). TVMV (Domier et al., 1986), three strains of PWV (Shukla et al., 1987), TVMV (Domier et al., 1985a, 1996), SCMV-MDB (Frenkel et al., 1991) and WSMV (Niblett et al., 1991). In contrast, the NAT strain of TEV (Allison et al., 1991), the five strains of PVY (Shukla et al., 1986, 1986) including the pepper mottle strain (Dougharty et al., 1985a), the D strain of ppv (Ravelonandro et al., 1988) and the SC strain of SCMV (Frenkel et al., 1991) had free N-tormini and gave good data on automated sequencing. Comparison of the maino acid sequences (Figs 5.1–5.8) show that all blocked coat proteins start with S, while the unblocked proteins have A or G at their N termini. The nature of the blocked N-terminus is assumed to be acetyl-S as found for JGMV (Shukla et al., 1987).

The coat proteins from distinct polyviruses vary considerably in size ranging from 251 emino acids for BeMMV to 332 amino acids for PPV-El Amar (Table 5.1). As shown in Figs 5.1-5.8 these size differences are largely due to variations at the N-terminal end of the coat protein. When the sequences are aligned for maximum identity these N-terminal regions range from 19 residues in BaMMV to 97 residues in PPV-El Amar. In contrast the C-terminal ends of the coat proteins vary in length by only one or two residues [Fig. 5.1]. Exceptions are SAPV, where the last 10 residues include a four residue repeat (MITHG) making it longer, and BaYMV and BaYMV where the C-terminal region is seven residues shorter than the average.

There has been some doubt about the true N-terminus of the cost protein of the rymovirus WSMV. Altompts to sequence it by protein chemical means have been unsuccessful prosumably because the N-terminal S residuo is N-acotylated as for JGMV-JG (Shukla et al., 1987). The cDNA sequence reveals five potential QS sites between NJB and the cost protein which would lend to the ganoration of cost proteins of 418, 322, 319, 307 and 288 amino acid residues with predicted molecular weights of 46.8 kDa, 35.7 kDa, 36 kDa, 34.3 kDa, 31.7 kDa respectively (Niblett et al., 1991). This range of molecular weights is in good agreement with the patterns of 42–47 kDa, 36 kDa, 33 kDa, 32 kDa and 31 kDa bands seen on SDS-polyacrylamide get electrophoresis (Brakke et al., 1990; Niblett et al., 1991). The smaller bands are considered to be further proteinase

et al., 1991). Examination of the partiol cDNA sequence for Wi sists of 418 residues and has a large N-terminal domain of 172 residuct) is very close to the putative NIb-CP junctions found in all a GDD sequence, whereas the second QS site (yielding a 322 residue p. 80) reveals that the first putative QS cleavage site (418 residues i genomes of members of the Polyvirus and Bymovirus genera (Fig. 4.2 with WSMV antibodies on Western blotting (Brakke et al., 1990; Ni breakdown products as they increase in proportion with time and all r residues apart to yield two forms of coat protein (Yeh et al., 1982) NIb-CP cleavage sites is found with PRSV where two sites occu NIb protein is not known, the occurrence of higher molecular we potyviral polyproteins. Since the extent of C-terminal processing of the NIb protein. This site is 79 residues downstream from the active the coat protein C-terminus) falls within a highly conserved sequent (Niblett ot al., 1991) and its comparison with the eligned sequences of folded on the surface of the virus particle. A second example of mul (Hollings and Brunt, 1901a) as might bo expected if the coat protein that WSMV particles are thicker (15 nm diameter) than most potyvir the GDD active site sequence are tolerated. Electron micrographs re forms of WSMV coat protoin suggest that upstream cleavages close

subgroups of sequences such as: (i) the alternating repeats of K a are enriched in K/E, K/D or related residuos as found in PVY, B) suggested that there are three major motifs; the long forms that have of WSMV having identical counterparts in SCMV-MDB. Similarly 26 (GSGSG sequences in LMV-O, PWV-K, ZYMV-C. WMV2, and particu K rich repeating sequences in the SrMV-SCH/DaYMV pair; and [tv soquences in JGMV, OrMV, SPFMV, PPV and SCMV-MDB; (iii) the A residues found in PRSV, PSbMV, TuMV, and MDMV-A; (ii) the P stream. The alignments in Fig. 5.1 also reveal small regions of sequ sequences have been eligned as follows: the N-terminal S, A or G resk proteins as a whole vary considerably in sequence. Key loatures in t in OrMV which contains no K residues but is rich in P and G residues C1YVV, PWV, ZYMV, SbMV, PRSV and TuMV; and the short form I rich sequences as found in PPV and SCMV; the medium length forms SCH. Hammond (1992) has also examined these N-terminal sequence: N-terminal 59 residues of BaYMV have identical counterparts in St WSMV have quito strong sequence identity with 32 of the first 65 resi Fig. 5.1 the N-terminal regions of the cost proteins of SCMV-MDB the SCMV-MDB/WSMV pair. As shown in the first block of sequence identity in the N-terminal region that are restricted to selected pair terminus, and the KKDK type sequences that occur 1-7 residues d the DAG aphid-transmission triplet within 5-12 residues from thi The data in Fig. 5.1 show that the N-terminal onds of potyvirus

In contrast to these veriable N-terminal sequences, striking ide across all sequences commences around the trypsin cleavage site (St et al., 1988b) beginning at the position equivalent to residue 30 is sequence KDKDVNAG in PVY-D. This sequence tdentity extends thr

Table 5.2. Summary of coat protein HPLC profiles

	Virus	Acronym	Reference					
8	Todacco etch virus	TEV-HAT	McKern <i>et al.</i> , 1990 McKern <i>et al.</i> , 1992a					
į.·	Potato virus Y	PVY·D、-10, ·18, ·43*	Shukla <i>et al.</i> , 1986, 1988a.c McKern <i>et al.</i> , 1992a					
:	Bean yellow mosaic	BYMV-GDD, G, G-81-1. F, K, RL7, S, BYMV-Scott	McKern <i>et al.</i> , 1993a MCKern <i>et al.</i> , 1992a, 1993a					
	Pea mosaic	PMV-204-1, -I, -Provvidenti	McKern et al., 1993a					
	White lupin mosaic	WLMV	McKern <i>et al.</i> , 1993a					
	Clover yellow vein	CIYVV-B, C81, LI, Pratt, Washington	McKern et al., 1992a, 1993a					
	Sweet pea mosaic	SPMV	McKern et al., 1993a					
	Passionfruit woodiness	PWV·TBM, -S*	Shukla <i>et al.</i> , 1988a,d					
	Watermelon mosaic 2	WMV2	Shukla <i>et al.</i> , 1988a Jain <i>et al.</i> , 1992 McKern <i>et al.</i> , 1993b					
	Moroccan watermelon mosaic	WMV-Morocco†	McKern et al., 1993b					
	Soyabean mosaic	SbMV-N SbMV-G1, G2, G3, G4, G5, G6, G7, VA.	Jain <i>et al.</i> . 1992 McKern <i>et al.</i> , 1993b Jain <i>et al.</i> . 1992					
		SbMV-Brazil, O. 12/18, 75/16/1, Wis	Jain <i>et el.</i> , 1992 McKern <i>et al.</i> , 1992a					
	Bean necrosis mosaic	BNMV-NL3, NL5, NL8, TN1	McKern et al., 1992c					

Bean common mosaic	BCMV-CH2, NI,1, NL2, NL4, NL6, NL7, BCMV-PR1, RU1, US1, US2(D,P&Z), US3 BCMV-US4, US5, US6, US7, US10	McKern <i>et al.</i> , 1992c McKern <i>et al.</i> , 1992c McKern <i>et al.</i> , 1992c
Peanut strips	PStV-Str, Blotch, Mild motle, China, PStV-T1, T2, T3, T6, T8,	McKern <i>et al.</i> , 1992a,b,c Kittipakorn <i>et al.</i> , 1993
Soyabean virus	PM, PN, 74	McKern et al., 1992a
Azuki bean mesaic	AzMV	McKern et al., 1992a
Blackeye cowpea mosaic	. B1CMV-Type, Wisconsin	McKern et al., 1992a,b,c
Peanut mottle	PeMoV-Poty-rape, Barnett	Kittipakorn et al., 1993
Cowpea aphio-borne mosaic	CABMV-Morocco	McKern et al., 1994
Papaya ringspot	PRSV-W	Jain <i>et al.</i> , 1992 McKern <i>et al.</i> , 1993b
Turnip mosaic	TuMV-	Haq et al., 1994
Maize dwarf mosaic	MDMV A-Type, Texas,	McKern et al., 1991b
Johnsongrass mosaic	JGMV-JG, KS1. 0	Shukia <i>et al.</i> , 1987. 1988a McKern <i>et al.</i> , 1990. 1991b
Sugarcane mosaic	SCMV-A, D, Isis, SC, BC, Sabi, MDB	McKern <i>et al.</i> , 1990, 1991b Shukla <i>et al.</i> , 1987, 1988a
Sorghum mosaic	SrMV-SCH, SCM	McKern et al., 1991b

^{*} Includes S. aureus V8 peptide profiles; all others are tryptic peptide profiles.

† Formerly the Morocco strain of WMV2.

DAAL	BD41/-SCC ACHE(POPIV	25./1868	
	BAYKY-TYP AADP LIDACKEDA-RIAAADGARFELADADRRKKEADRVEAA-RVKKAADAALKPVWLT	PAYKY- TAD	
DDAKKKADALYKOHOLADAKKKA 79	ASSG·····INDACATTAEATAGAGE>AAAKAGRD··ADA··KKK··AD·DEAAERORGOAAKKKADDDAKKKADALYKGHG ADAKKKA	PLKA-SCH	
CAXICAGSGSKGTGGSFT 65	\$\$~~~~~~QEASFA\$G\$G\$\$G~~~~~\$G~\$G\$Z}AGG\$G\$Q\$QA~AQTG\$HKV\$\$VXA~GLDTG~~~GJXTGGG\$G\$G\$GTGG\$FT~~	VSRV	
GSGARGJASGSGSGTEGVRTG. 79	SS INDIAGREGESE-SO-GITPPHICSCARPAISCUGSGSGIGGETGVICZGARI-GSGIGTGSGAIGGGSGSGSGSGTEGVHIG.	BCH - MOB	
A26320 CLOLL GLACHERYS - BI	ABEREBEE ENDACAPLAITAPAATSPILGFP-PVICPAPRT (APHLHPI) TPATIGPATKPUSGYSGOCLGJI-GTYGHIDAS	14. 644	
ESY-GRDTSKEK 65	SSERTEFXDACADPPAPKPKHIFSPPTITEVIDPEDPXDAALAAAXXQPATIPESY-GRDTSKEK	SBINAS	
S-AKG/E (B	IDE 1 JLOTEK WETK KKODI EGIPPOEMIPTOP GPSDKGKEV	SPLV	
	УD 94D AGG SSR PPAPL V	OFNV	
-S0TKO36 55	SGHTOASKOKSATPAANDTASGCGXFVCTTATADNKPSSLVTSVA-OGT-SQTKQGG	Dr-7301	
	AGE MOACOX IDAOKEAEKKAKEAKEAKEAKOKE I-KEKS	W-Z-HOH	
	MANY-OLED AGEILONDERGECK-GAEKEKKEREKAEKEREAKKGEAFKKGK	PUNY-Queb	
DV 27	AMETL WACAS TSTOASBATRPEAAT	Ali)-ee	
	AGDE TRODE ARRKEEEDRKKREES IDASOFGSSSDXXXXKKK	14-11056	
	nd	PLSY-P	
	SGSGHPPLPVVDAGADIEXO-XXDX-SSRGRDPEHXE[EXNX;	BOAR-ALA	
	SSKKEG E R-DALIZO TREK DKGK	BONY-HLB	-
	SGXEKE6 DHDAD GDP XK\$1-SSSKG	H-, MAGS	ì
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- FRIP IEDDCKLK PSGA-RIP-SSAJOGNISVBATROVNAJCI (KIPLNICKSKUZICI) NYAJOPONAKS		99F - VISTANDIADADADADADADADADADADADADADADADADADAD		-	•	1) CAGLIXXARALIKO DILATVURUKS - ICADGASID I HOTHTI I YAKSAH d'ILKYASASA IVAGA ALGISGAGAGYAZIYI IMIGOGS 151 ATTA TARAKI HAMBASHI INDINE - TARAKI INDINANI MARKAH GISAMASHI ALGISGAGAGYAZIYI MARKATI INDINANI MARKATI INDINANI MARKATI INDINANI MARKATI		_		_	TEL - OLIA SYAKIBATEN LUKKALESI TOLOGONYO TIYOTHIIN KOMMANAKA THEKKALIYOTYAN SALAYAYAKAMI (MILO-US- AA - OLIMBILANI KATIMIN KATIMI TOLOGONYA SITIOTHIIN KATIMIK INDONETYATI SALAYAYA TAKAMI KATIMIKANA KATIMI		_		111 - GILD SERBY SERVE FOR THE FIGURE SALE FOR THE FOR	_	•			_	_			

Rymovirus genus and BaYMV and BaMMV are species of the Bymovirus genus. The data sources are Fig. 5.1. Multiple alignment of the amino acid sequences of the coat proteins from 31 distinct represent invariant rosidues; and lower case letters represont nearly invariant residues. In the first block listed in Table 5.1. A consensus sequence is given at the top of the liguro where: uppercase letters polyviruses. The first 28 are from members of the Polyvirus genus; WSMY is a species of the sequences the listed sequence order has been changed to highlight the similarity between the

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ELMY-25C DICH-JANVZZWASANIERICEZH-H-UNDIG-18DIG-18DIG-19-CHARANIEN H-GEZÖKZEZHASTANA (1918) DICH-18-CHARANIERI SPHYTRS DIGHTCTTANCLHVACTICHGISP-1-14C24FA-PD-5-CEGO-TIZZIKALLDHAV-PITRQIAITHSVVAKATICHBRRI-CAYMPRI

TO CAUTON: 17/10/JCCHHG15-OCAAE-HOVINGIOSGC-- GAPTLASLSS FI VHARHHGGLRH I MXXYSDETVLLIT-HXXLVANUSKCI DISA-TPVHSGLNING [[ESCSP-H-LHGHJ]H-KD-K--DECR-VFPLKPV][LKS--P]] RDJMHH IDJAEAY ILYRUS--CRTPPRI CODA-SICHHOLARASI (18325-18-1 NODMAN-10-6--DLOS-22AFRI SLAVEN SASTE SOLYEN LOLAGI - ODARA (ODARA) - ODARA (ODARA (ODARA) (ODARA (ODARA (ODARA (ODARA (ODARA (ODARA (ODARA (ODARA (ODARA)

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201-100

OF CK-KATHONIN B-DSJPPACTIVETING NO. 9-0

F SUHU-PI

TAMA-BAND TORN -OTTINGTARALIBATAN-A-TO-CECOM-NO-2--CECOM-ELS TAN IDHAX-- BILBOTHAN BOARENTIEKBADS-SALMANA

DECH-RII HHAIMACI ENGISP-D-I HEBUNH-ND-G--HNOS-EFPLKPINKKAK--PIL GCYWDFSDAAL A I IERGKLD-EFFRIR) DICH-IVWSSLEACHERGCSP-R-INSWIM-ND-G--GEOR-IFFLOWIERAL--PIFRGINSVESSAALAFIETERST-EKTEDRI

- DEQI - EYPLOPYLEAAO-->11 AQIMAHFSHAAEAY IL CAUS- GLIMPAT

dern yftyrgenangeieheetry-y-ragarin-ad-2--deog-Cebysdilehye-bet eqinariekaeteeatieeghte-kaard OHEN-CALASCENCATIENSES 49-N-ENGRATH-NO-G--EEGV-EYPLKPCCCCNG--PVERGINKHSGDACAYTECGASI-EVF----

CVEN-GANTHOTANGLIVELLARIZA-D- I SPLANM-AD- C--ELOA-DALIZALICAT--b82-BOLMAHISHANTVITABAVI-CHAMBAL-CHAMBA DDCM-A|| HUCHMANCLONGISP-D-VHANVAM-MO-O-OLOV-IYPLCFMVERAK-O-PILROHMV.150KALAY1EHRHSE-RPYMYNY dgck-gannrzenarcidhgisp-o-knzarm-hd-g--dio]-exalbkrenke--blikbahhh solleni iehrhst-githbkr

DEAH-CLANAG INANC IDHO 184-6- YACAMAN-AD-6--EE OT-ELALI WALIONE-- bIT JOINHHI CODH - CAANAGAMAAC I EHGA 59-0 - I NGAMAN - ND - G - - HEGA - EALMAN I ALMAN THEND J

SDAJEAT I LARUSE - S PI HPÄY

abbald sivemiliaty two sibbald - nyvęzniands - des proje - d - ck - nipaska - q - st sa si spanjanias - koci

DSRH-G11+ACLHVALLIEHD185-D·LCGCH1H·HD·G··EEGV: TYPLKP ILOWK--PT FRQINSHT1EVACATILCÄULI

52.80 - N. 4 PARAPERTY TOTEKATETI TYTYL I LEGIZE I BALLOS I GARANINI I STORICAS I STORICA I LEGIZET STATE STORICA S XAPY-NTAL GLLAHLKYCKLARYAFOFYEYFSK-FPYRAKKATAGHKAAAL-AHYHFRHFGIDGWVATTSLHTERHTAIDYNGHHISLLGATHGA 300--2418 ÄRYA-SHA _ CFOBRICOF2FYBJ-FD1.1F11.1E1.1E18BEKYIATENIYBEKYIE-19VANDELIZHTET HESTELEHELDHIDDHIDHELICARCX consensus gl-rnl-o--laryalbiye--s--lp-carea--qnirab----------fgldg-y----enteRht-Oy--n#i-lib----PSLAV-PI ALDIN ADVSLARTGIDITLIIAC 1242/91AAFONKAAAI KGESHSLFCLUGNYCIGILUTLRHIJCOHONHHILLDAAA TABRO TO THE AREA TO THE BIND OF THE SECOND TO THE SECOND TO THE SECOND TO THE SECOND THE SECOND THE SECOND TO THE SECOND GRL RC-NOWGLARYAFOFTETTSA-TITHRAREA-NONCAAAL-VGTONTLFGNOGGGSTOEEN JE RHIAAUVBDNWITTG/HGCLI GL GR HL ICHSLARYA (D FYEIVI SR - IP I FIRE AH I GWKAAAL - RGAKHKL (GIOCGWG) I VEH I ERNI I EGVEKKWINLLGWOSI GLOBA_TOTHLARFATOTYLVISL-I>JAJANLAHTOHCIAAL-RGKOSCLFGJDCXVJ IGQEOTLBHJJGDGHKHHHSITGISH Cikrhi id isi ai aaof ilanik. Indharlahmmatarat - i misikhi chdra ahkelinia kulaedahicanati caha GLA SHI KUCULUCTAFO TEEVISK - TSDRAKTAFAGYXAAAA - SA YSSKLT GI CGUYAAT SEHTERII AKOUWOAFATALGAGPPO GLLRN: ADRELARTAJ DE YLVISA - YYNAHEALAGNKAAL OGENOTY INTERNATY OF LEAL HEST INTO THE TRANSPORTY PORTY PROPRENEUR. AS TAIL LEADY TO THE TREE THE TRANSPORT P STANDTHE THE TRANSPORTE SHE THE TRANSPORTY PORTY PROPRESSED THE TRANSPORT PROPRESSED THE STAND THE TRANSPORTY PROPRESSED THE STAND THE TRANSPORTY PROPRESSED THE STAND THE TRANSPORTY PROPRESSED THE TRANSPORT PROPRESSED THE TRANSPORTY PROPRESSED T GLLRVLJCXKLARTA FOT YEVHAC- I STJAN SAVADKKAAAL - SHVTKKI FOLOGHYAT I 540 IERHIJRDAREKHHSLI GWDPU Cilhrendese aktaidi il irta - Tehrare akkamcakai - restumiceder-ge ssekterhaadysehvistrektaet C. Garl 1973 tarkafol 14 (188- Sdabakeurdakatan-80 soluhi CT) dhactahen (Culacarabansi Toroga · AHVH I RL I GLOCYVALI SCHI CAHLARC'H QHHEILL GAI SGO - SCAMMCT I CYDCHI ZINZEMIE BHYYBDAHOHUNI I CHCD bo

occurs 96 residues upstream and is shown in Chapter 4 (Fig. 4.2). PRSV is also reparted to use been obtained by HPLC peptide mapping as listed in Table 5.2. In this ligure the N-terminus of th second site 20 residues upstroam from that shown here. WSMV coat protein corresponds to the second of the live OS clossvago sitos used. An additional : SCMV-MOBIWSMV and SMV-SCHIBaYMV pairs. Additional Information on comparative sequences The additional sequence is also shown

some contain additional Cs. The conserved AFDF sequence (equivalent to menlous plant viruses, the potexviruses, carlaviruses and closteroviruses half. Most sequences contain the C residue equivalent to 119 in PVY-D and servation in the C-terminal half of the cost protein than in the N-terminal polyviruses. As shown in the consensus sequence in Fig. 5.1, P residues 12 of these also conserved in those of the mite- and fungus-transmitted coat protein sequences of the 26 aphid transmitted potyviruses with in Fig. 5.1. There are 56 amino acid residues totally conserved among the lighting the conserved regions is shown along the top of each block of data 199–202 in PVY-D) is also found in the coat proteins of three other filafeature frequently among the conserved residues and there is greater conthe Clerminus of the coat protein. A consensus sequence motif high

Coat proteins of virus strains

coal protain paptides from four strains of PYY have also baen established strain of PVY sequenced by Dougherty et al. (1985a). KPLC profiles for PupMoV is a distinct potyvirus from PVY and from the popper mottle complete genome of an authentic isolate of PopMoV. These data show that et $al.,\,$ 1985a). As shown in Fig. 5.2 it has vory high sequence identity with did not have a blocked N terminus. The amine acid sequence of a pepper Vance et al. (1992a,b) have sequenced the coat protein (Fig. 5.1) and 1972), should be considered a stroin of PVY (Shukin ot al., 1986). Recently that PapMoV, originally described as an atypical strain of PVY (Zitter, mottle virus isolate of unreported origin has been determined (Dougherly contained only a single C residue (at position 119) and, where examined regarding the first three amino acids (Hay et al., 1989). All PVY strains residue shorter having a deletion at position 25 (Shukla et al., 1988c) contain 267 amino acid residues except for that of PVY-18 which is one viruses (Toble 5.1) and these will be discussed in detail in this section of the aphid-transmitted potyviruses and two fungus-transmitted potythe other strains of PVY and on tho basis of this homology it was suggested plants and different parts of the world are shown in Fig. 5.2. These CPs The coat protein sequences for 22 strains of PVY from different host The sequence for PVY-NZL is incomplete as no information is available Primary structure data are also evailable for multiple strains of 17

PVY° ('common') isolates and are more diverse. Thuy can be further grouped into four clustors: the four Australian isolates (D, 10, 18 and 43); the strains, with the exception of PVY PupMa, were classified as typical as typical PVY" ('nocrosis') isolatos (Van der Vlugt, 1993). The rest of (PVY-N11, N12, Jp, T. GO16, NZL, Hu and Russ) have been described tho six isolatos ffr. US. 02. 03. 04 and Ch): strain I on its own: and tho they fall into two subgroups. Most of the viruses in the first subgroup Van dor Vlugt (1993) has analysed these 22 sequences and shown tha

> sion (Xiao et al., 1994). As shown in Fig. 5.3 the PMV-I coat protein show of al., 1993a). The complete sequence of PMV-I has confirmed this conclu of BYMV and CIYVV have been compared with those of peu mosaic an in Fig. 5.3. The CIYVV-30 strain is two residues longer than the others sequences for the coat proteins of three strains of CIYVV are also show acids) and many of the substitutions are shared by other strains. The reveal 15-33 differences between them. All are the same size [273 amin differences between these two strains. very high sequence identity (97%) to that of BYMV-CS with only eigh white lupin mosaic viruses as summarized in Table 5.2 and revealed the fer from each other at 21-22 positions, HPLC profiles for several strain isolate has an additional C residue at position 181. The CIYVV strains dil the latter viruses are strains of BYMV, not distinct viruses (McKer. baving a double insertion, VG, at positions 29 and 30. The New Zealan The sequences for four strains of BYMV are shown in Fig. 5.3, and

show that BNMV-IN1 is very similar to these three strains (M::Ker and mutants of TVMV (Auroya et al., 1990, 1991). The HPLC profile be expected to abolish aphid-transmission as found in the NAT strai mulation at the third position of the DAG triplet at residue 11 which woul with only five differences between BNAV-NL3 and NL5 and 7-10 di formerly the serogroup A strains of BCMV) are very similar to each other BNMV and BCMV, are shown in Fig. 5.4. The three strains of BNM' ferences between these two strains and NLO, BNMV-NLO has a G to The sequences for strains of two other viruses that infect legumen

sequences differ at only two positions and are as similar to the BCMV an et al., 1991a) and PSIV-Motch (Cassidy of al., 1993). The two PSI protein sequences of BICMY-W (Khan et al., 1993), PSIV-Stripe (McKor and NY15 (Khan et al., 1993) are shown in Fig. 5.4 along with the cou aphiel trunsmission since this mutation was without effect on TVMV sit W. PSIV-Stripe or PSIV-Blotch. BCMV-NLL and NY15 have a G to S mutstrains of the one polyvirus, BGMV (McKern et al., 1992b,c). (IPLC profile NL1, NY15 and NLA. Thus BCMV, BICMV and PStV are considered to b specific mulants (Afreya et al., 1991). first position of the DAG triplet of PStV-Stripe should have no offect c tion at the third position of the DAG triplet. The mutation D to N in the residue at position 216 in DCMV-NL4 is not shared by NL1, NY15, DICM1 tional C residuo at position 26 in the N-terminal region. The additional DICMV sequences as are the sequences of the accepted DCMV strain this close relationship (Table 5.2). BCMV-NL1 and NY15 have an add for another 10 strains of BCMV and eight strains of PS(V have confirme The coat protoin sequences of BCMV-NL4 (Votton et al., 1992b), NI

broolve residues in the DAG upbid transmission signal in the N-termin few differences botween the strains of each virus. Many of the difference WMV2 and thrae strains of ZYMV are shown in Fig. 5.5 and reveal ver Cont protein sequences for three strains of SDMV, three strains

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